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Studies for optimization of conditions for reducing Aflatoxin Contamination in Peanuts using Ultraviolet Radiations

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Abstract: The aim of present study was to optimize the radiation dose for maximum reduction of aflatoxin content by varying the distance between the contaminated peanut samples and the UV-C light source and duration of exposure to UV-C light. Studies were also undertaken to evaluate the effect of UV-C exposure on the various quality parameters of peanuts. Two different studies were conducted in a sterilized wooden box fitted with a 15 W UV-C tube in the centre at the top of the box, where the contaminated samples were placed at a distance of 15 cm and 30 cm respectively from the UV-C light source. The duration of the exposure was varied from 2 h to 12 h. The fungicidal activity of UV-C radiation was found to be more pronounced in peanuts exposed at a distance of 15 cm. Maximum reduction of aflatoxin concentration in both the studies was found after 10 h of exposure. In the studies, conducted at a distance of 15 cm, aflatoxin concentration reduced to 99.1 % (350 ppb to 3 ppb) with decrease in fungal count to < 10 $cfug^{-1}$and at a distance of 30 cm, reduction of 97.4% (350 ppb to 9 ppb) of the aflatoxin content with< 10 $cfug^{-1}$ of fungal count was observed. However, the quality parameters including nutritional values and physico-chemical parameters of peanuts remained relatively unaffected (within the national and international regulatory limits). The present study thus provides a validated practical method of using UV-C irradiation to reduce aflatoxin contamination in peanuts, intended to be used for the purpose of food and feed.

Keywords: Aflatoxin, *Aspergillus flavus*, Peanuts, UV-C irradiation.

Full Length Original Research Paper

Introduction

The presence of mycotoxins in food is one of the issue of food safety since they are the potential source of health hazard. Mycotoxins are biochemicals produced by filamentous fungi, mainly members of genera *Aspergillus*, *Fusarium, Alternaria* and *Penicillium*[1, 2]. Mycotoxins are produced at all the stages of agriculture i.e. at the time of pre-harvest, harvest and post-harvest and also during the storage[3]. While species of *Aspergillus* and *Penicillium* are generally found to contaminate food and feed products during drying and storage conditions, *Fusarium* and *Alternaria sp*. can produce mycotoxins both before or immediately after harvesting [3, 4].

The production of mycotoxins is dependent upon various factors, mainly the agricultural practices, environmental and storage conditions. The agricultural practices include soil conditions: moisture, pH, temperature, nutrients, selection of seed variety, seed density, crop type and suitability of equipment used for harvesting. The environmental conditions include water activity

i.e. moisture and relative humidity present in the grains, duration of exposure of the product to stress conditions and interactions with other species such as microbes, insects, animals etc[5]. Storage conditions involve high temperature, high humidity and light. More than 250 mycotoxins have already been identified, but relatively a few of them are considered to be hazardous to human as well as animal health. In order to ensure the quality and safety of food products, it is important to monitor the mycotoxins such as aflatoxins, ochratoxins, fumonisins, trichothecenes and zearalenone in different food products[6]. The regulatory authorities in the developed world especially EU countries, have put strict regulations to control the presence of mycotoxins in all imported food products.

Aflatoxins are a group of potent carcinogenic, mutagenic and teratogenic mycotoxins. They cause acute toxicity. While aflatoxins B_1, B_2, G_1 and G_2 are found as contaminants in the food, derived from land farming, their metabolites like M_1 and M_2 occur as contaminants in food of animal origin including meat and milk. Thus, humans are at a high risk of getting exposed to different types of aflatoxins depending upon the type of foods being consumed by them. Thus, vegetarians are likely to consume aflatoxin B1, B2, G1 and G2 from the agriproducts and M1 and M2 from milk and milk products and the non-vegetarians are likely to consume, in addition to the above, the aflatoxins M1 and M2 from meat and poultry products as well. Regulatory agencies such as food and drug administration of US (USFDA) have specified guidelines on acceptable levels of aflatoxins in the food for humans and in feed for animals. The permissible level of the total aflatoxins in food products for humans is 20 ppb as per USFDA norms and 4 ppb as per the EU authorities. The average level of aflatoxin in India is reported to be about 100 ppb in peanuts (Survey by Central Food Technological Research Institute (CFTRI), Mysore) and it is about 170 ppb in peanut oil [7]. This clearly shows that it is getting difficult for the suppliers to capture the global market (especially of EU) for certain food products known to be prone to contamination by aflatoxins. Peanuts are one such major crop vulnerable to contamination of aflatoxins of the type B_1, B_2, G_1 and G_2. Peanut is considered to be a cash crop in India due to its importance both as a food as well as a source of vegetable oil for humans and as a feed (oil cake) for animals[8]. The fear of the loss of crop due to fungal invasion leading to the contamination by aflatoxins is the main cause for low levels of productivity of peanuts in India. Further, the presence of high levels of aflatoxins in by-products (HPS-grade kernels and deoiled cakes) from vegetable oil industries has been the cause behind the rejection of export consignments of these products. Infact, for quite some time now, the exports of peanuts as well as the products derived from peanuts of Indian origin have almost been banned, especially in EU & certain other countries.

Presently various efforts are being made to develop and adopt appropriate pre & post-harvest management practices in order to control the aflatoxin content in various crops. The experience has shown that it is easier said than done when it comes to making a particular technology work successfully for controlling aflatoxins in peanuts. Several physical methods like heating, roasting, frying, boiling, baking, drying and roller drying to the safe moisture levels (<7% for peanut kernel) have been reported for detoxification of aflatoxin contaminated peanuts etc.[9,10] Most of the presently available

technologies do not seem to work successfully to bring down the levels of aflatoxins to comply with the desired standards of quality set by EU authorities. The only solution appears to be the strict adherence to the good agricultural practices. It may be noted that utilization of food grains already contaminated is as much a big challenge as controlling aflatoxins during agriculture. The big question which is difficult to answer is, what to do with the crop already contaminated with aflatoxins? Destroying the crop or letting it divert to the local consumers, if not suitable for exports would never be approved as an acceptable option. Therefore, methodologies need to be developed not just for controlling but also for decontamination of the already contaminated crop. The effect of various techniques such as roasting, use of gamma rays, sunlight, microwave heating on elimination of aflatoxin have also been studied[11-16]. The use of ultraviolet-C (UV-C) radiation having germicidal effect has widely been reported for destroying the pathogenic fungi including aflatoxins & other fungal metabolites that may contaminate food products. A number of in-vitro studies have revealed the efficiency of UV–C radiation on microbial inhibition[17,18]. Germicidal effect of UV-C irradiation at 250 mW/cm² for controlling the microorganisms i.e. *Pseudomonas, Streptococcus, Acanthamoeba, Candida* and *Aspergillus niger* in 20 minutes has been reported by Gritz et al[19]. Marquenie et. al[20] has established the inactivation of *Botrytis cinerea* and *Monilinia fructigena* in strawberries and cherries with UV-C treatment at 0.50 and 0.10 J/cm² respectively.

Besides inhibiting undesired microbial growth, the use of UV-C irradiation has also been reported for the degradation of aflatoxins in food products. irradiation has been mentioned in a number of studies. Studies have also been conducted to monitor the degradation kinetics of aflatoxin M1 (AFM) in aqueous medium following UV irradiation (365-nm) to determine loss of the toxin and development of reaction products. Degradation of aflatoxins by UV irradiation follows first order kinetics[21-23]. UV irradiation from sunlight has also been reported for detoxification of aflatoxins. Studies undertaken by Shantha and Murthy[24] have shown that sunlight destroys 83% of the aflatoxin added to casein and 50% of the aflatoxin added to groundnut cake flour. Detoxification of aflatoxin B1 by solar radiation in a coconut oil in especially designed pilot plant was established by Samarjeeva et al.[25]. The effectiveness of this technique for the treatment of peanuts has not been reported. Inspite of the fact that there have been efforts to control and decontaminate peanuts from aflatoxins, fool-proof technology is yet to be developed.

In order to explore the feasibility of using UV-C irradiation for decontamination of peanuts contaminated with aflatoxins, the present study has been carried out using a UV sterilizer consisting of a UV-C tube. The studies were carried out by varying the distance of the contaminated peanuts from the UV-C tube and duration of exposure of contaminated peanuts to UV irradiation. Besides studying the effect of UV-C radiation for decontamination of aflatoxins in peanuts, studies were also conducted to evaluate the effect of radiation on the nutritional components of the peanuts including the quality of extracted oil.

Materials and Methods

(A) Materials and Equipment:

(i) Chemicals: All the chemicals and reagents (petroleum ether, sodium thiosulphate, sulphuric acid, boric acid sodium hydroxide, ammonia, hydrochloric acid, dextrose, ethyl alcohol, potassium hydroxide, acetic acid, chloroform) used were of AR grade and were procured from 'SD-Fine chemicals, India'. HPLC-grade methanol and water were procured from 'E-Merck'. Chloramphenicol yeast glucose agar (CYGA) was procured from 'Himedia, Mumbai, India'.

(ii) Germicidal UV Tube, a low-pressure mercury vapor discharge lamp in a tubular glass envelope, with 15 W power and 254 nm wavelength providing UV-C radiations was procured from local sources.

(iii) UV Sterilizer (60 X 60 cm) fitted with 15 W UV-C tube & having slots at distances of 15 cm & 30 cm for the trays, was fabricated in-house shown in Figure I a & I b.

(iv) Enzyme-linked immunosorbent assay (ELISA) kit (Ridascreen Aflatoxin Total) for the estimation of total aflatoxin was procured from 'R-Biopharm, Darmstadt, Germany'. The kit consisted of 96 well microtitre plate, six standard solutions (o ppb, 0.5 ppb, 1.5 ppb, 4.5 ppb, 13.5 ppb and 40.5 ppb), peroxide conjugated aflatoxin, anti aflatoxin antibodies, substrate containing urea peroxidase, chromogen (tetramethyl benzidine), stop reagent (1M sulfuric acid) and dilution buffer. ELISA reader, model no.680 from 'BioRad, USA' was used for determination of aflatoxin content.

(v) Collection of Peanut samples: Aflatoxin free post harvested peanuts of G10 variety were procured from 'Junagarh research farm (Gujarat State, India)'. Wide mouth PET jars sterilized by gamma radiation were used for storing the samples of peanuts. The collected samples were transported to the laboratory in an ice box consisting of ice packs to prevent any changes in the microbial flora of the samples.

(vi) Fungus used for inoculation: Aflatoxin producing Strain of *Aspergillus flavus* ITCC 1717, was procured from 'Indian Agricultural Research Institute, Delhi'. Fungus was grown on Chloramphenicol Yeast Glucose Agar (CYGA) at 28 ± 2 °C with a photoperiod of 10 h per day for 2-3 days. After incubation, the plates were stored at 4 °C until further use.

(vii) Fungus inoculated peanuts: Surface sterilized peanuts, dipped in a solution of 0.1 % mercuric chloride ($HgCl_2$) for 1 min and washed twice with 'Milli-Q' water were inoculated with *A. flavus* fungal spore suspension at a rate of 10^6 spores/ml. The inoculated samples were kept at 28 ± 2 °C for 4-5 days. Following the fungal growth, peanuts were mixed thoroughly in order to obtain homogeneity and stored in polyethylene (PE) bags (88 µm-thicknesses) which were then sealed under ambient conditions of atmosphere & kept under refrigerated conditions.

B) Methodology

(i) Exposure to UV-C radiation

[Figure Ia & Ib] shows the UV sterilizer fitted with 15 Watt UV-C tube at the centre on the top. Two different experiments were carried out whereby 2 Kg each of peanut samples were spread in a layer not more than 3 cm thick in two different trays and were exposed to UV radiations. The exposure of the samples was done by varying the following parameters:

1) Distance from UV-C tube: The exposure of the contaminated samples was done by placing the samples at two different distances i.e. at 15 cm and 30 cm from the tube.
2) Duration of exposure: From 2 h (108 KJ/m^2) to 12 h (648 KJ/m^2) at 15 cm distance and 2 h (72 KJ/m^2) to 12 h (432 KJ/m^2) at 30 cm distance at time intervals of 2 h at both the distances.
3) Peanut samples were exposed for different time duration i.e. 2, 4, 6, 8, 10 & 12 h at both the distances. In order to measure the precision of the results, the sample was exposed five times at each of the time interval. Thus a repeated 30 irradiated peanut samples at one particular distance from the UV-C source were obtained and a total of 60 irradiated peanut samples were collected by exposing samples at a distance of both 15 cm and 30 cm from the UV source. All the 60 irradiated peanut samples were analyzed in triplicate for aflatoxin content, fungal count and various physico-chemical parameters.

(ii) Studies for Enumeration of Total Fungal Count (As per IS 5403, 1999)[26]

The total fungal count (TFC) of the contaminated peanuts samples was determined before and after irradiation. 10 g each of the unirradiated (control) and irradiated ground homogenized peanut sample was mixed with 90 ml of 0.1% peptone water. After proper mixing, samples were serially diluted upto 10^{-7} dilutions using 0.1% peptone water. 1ml of each dilution was transferred into three sterile petridishes (90 mm of size). About 15-20 ml melted media (Chloramphenicol Yeast Glucose Agar) was poured and mix properly by rotating the plates clockwise and anticlockwise. Plates were incubated at 25°C for 5 days. The data presented is the average count in three Petri dishes for each sample. Plates were finally, incubated at 28±2°C for 5 days. Colonies on the plates were counted with the help of Quebec colony counter and then calculated in terms of colony forming unit per gram (cfu/g) of sample.

(iii) Studies for determination of Aflatoxin content

Aflatoxin content in the samples was determined as per the procedure described in the validated ELISA kit for Ridascreen® Aflatoxin Total, both before and after exposure to UV-C radiation.

(a)Extraction of aflatoxin content:

Accurately weighed 2 g of crushed sample each of both unirradiated and the irradiated contaminated peanuts was taken in individual iodine flasks and extracted using 10 ml of HPLC grade methanol/distilled water (70/30, v/v) for 10 min at room temperature on orbital shaker. The content of each flask was then filtered through Whatman filter paper No. 41. The filtrate (100

µl) was diluted with 600 µl of phosphate buffer of pH 8.0. This extract was further used for the determination of the aflatoxin content.

(b) Determination of Aflatoxin content:

Six standard solutions (consisting of all four aflatoxins i.e. B1, B2, G1 and G2 with total aflatoxin concentrations between 0 ppb to 41 ppb provided in the aflatoxin determination kit (diluted in the ratio of 1:10 with dilution buffer) and extract of the peanut samples (50 µl each) were dispensed into separate wells in duplicate. An equal volume of diluted enzyme conjugate and diluted antibody solutions were then added to all the wells. The reagents were properly mixed by shaking the plate with plate centrifuge machine and the plate was then incubated for 30 min at room temperature (25 °C) in dark. After incubation, liquids were poured out of the wells and the microwell holder was turned upside down and tapped thrice against absorbent paper to ensure complete removal of liquid from the wells. All the wells were filled with 250 µl distilled water and the liquid was poured out again. The washing procedure was repeated twice. 50 µl of substrate and 50 µl of chromogen was added to each well. The solutions were mixed by shaking the plate manually and incubated for 30 minutes at room temperature (20 – 25 °C) in the dark. Finally 100 µl of 1 N H_2SO_4 was added to each well to stop the reaction. The solutions in the plate were again mixed properly and finally the absorbance was measured at 450 nm using ELISA reader. Due care was taken to measure the absorbance within 30 min of addition of the stop solution.

(iv) Studies for evaluation of the effect of UV-C radiations on the nutritional components of peanuts:

The different nutritional components in the irradiated peanut samples were evaluated using the procedure as given below:

a) Fat Content (As per AOAC method 948.22, 2005)[27]:

20 g sample of both control (unirradiated) and irradiated crushed peanut sample was extracted with petroleum ether in a Soxhlet apparatus for 16 h. The ether was evaporated and dried residue (at 95-100°C) was weighed. Fat content was calculated as follows:

Fat (% by mass) : $[(W_2 – W_1)$ X 100] / W

where:

W = Weight of sample in g; W_1 = Weight of empty flask in g; W_2 = Weight of flask with extracted fat in g

b) Protein Content (As per AOAC method 950.48, 2005)[27]:

700 mg crushed peanut sample was treated with 5 g of digestion mixture (0.5 g $CuSO_4$. $5H_2O$ in 5 g K_2SO_4) and 25 ml concentrated H_2SO_4 for 3-4 h until the sample became colourless. This was cooled and 20 ml deionized water was added followed by 25 ml NaOH (40%). The sample was then distilled and the ammonia liberated was collected in boric acid and titrated with 0.1N HCl. A blank was prepared and treated in the same manner except that the tube was free of sample. Protein % was calculated according to the formula:

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Total Protein (% by mass):[14 X (S – B) X 6.25 X 100] / W

Where:

B = Volume in ml of Standard NaOH used to neutralize acid in blank determination; A = Volume in ml of Standard NaOH used to neutralize acid in test sample determination; W = Weight of test sample in g; N = Normality of NaOH

c) Carbohydrate content (As per IS: 4706 (Part 2)-1978)[28]:

This method is also called as the Lane Eyon method 20 g of sample was refluxed for 2.5 h with 200 ml distilled water and 10 ml of concentrated acid. The solution was then cooled at room temperature and 5 ml each of potassium ferrocyanide [$K_4Fe(CN)_6$] and zinc was added to it and the volume was made upto 500 ml with distilled water and filtered. 50 ml of the filtered solution was neutralized with 50% sodium hydroxide (until the solution become dark purple) and diluted to 100 ml with distilled water and titrated with the mixture of Fehling's solution using methylene blue indicator solution until the colour of the Fehling's solution changed to brick red. Similarly blank determination was also carried out at the same time as above using the standard (Std.) dextrose solution with the Fehling solutions.

$$\text{Carbohydrate: (\% by mass)} = \frac{\text{Titre value of Std. X 2 X Vol.made X 100 X 0.93}}{\text{Titre value of Sample X Sample wt. X 10}}$$

(v) Studies for evaluation of physico-chemical properties of the oil extracted from the UV-C irradiated samples of peanuts

The physico-chemical properties of the peanut oil (Acid value, Saponification value, Peroxide value & Refractive index) were evaluated as per the AOAC Methods (18th Ed. 2005) as described below:

a) Acid value (As per AOAC method 969.17, 2005)[27]: 5 g of extracted oil was mixed with alcohol-ether mixture and phenolphthalein indicator solution and the mixture was titrated with 0.1 N alcoholic potassium hydroxide solution until the pink colour appeared and persisted for at least 10 s. Acid value was calculated as:

Acid Value (mg KOH/g of oil) : 56.1 X V X N / W

Where:

V = Volume of Standard KOH in ml; N = Normality of KOH solution; W = Weight of Sample in g

b) Saponification value (As per AOAC method 920.160, 2005)[27]: 5 g of oil was mixed with 50 ml of alcoholic potassium hydroxide solution in a conical flask. The flask was connected to air condenser and boiled until the sample was completely saponified (as indicated by absence of any oily matter and appearance of clear solution) and cooled. The solution was titrated with 0.5 M hydrochloric acid using phenolphthalein indicator solution. Blank determination was also carried out at the same time as above with 0.5 M hydrochloric acid and value quantified as per the following formula.

Saponification Value: 28.05 (B - S)/ W (mg KOH/g of oil)

Where

B = Volume of Standard HCl required for blank in ml; S = Volume of Standard HCl required for sample in ml; W = Weight of sample in g

c) Peroxide value (As per AOAC Method 965.33, 2005)[27]:

5.0 g of the oil sample was properly mixed with 30 ml of the acetic acid-chloroform mixture. 0.5 ml of saturated KI solution was added to it and the mixture was allowed to stand for one minute with occasional shaking followed by addition of 30 ml distilled water. The mixture was titrated with 0.1 M sodium thiosulphate solution with constant and vigorous shaking until the yellow colour almost disappeared. 0.5 ml starch solution was added to it and titration was again continued with vigorous shaking to release all iodine from chloroform layer, until the blue color just disappeared, Similarly, blank determination of the reagent was also conducted in the same way. Peroxide value was calculated by the following formula:

Peroxide Value (mEq/kg): (S – B) X M X 1000 / W

Where:

S = Volume in ml of Sodium thiosulphate solution used up by sample; B = Volume in ml of Sodium thiosulphate solution used up by blank; M = Molarity of Sodium thiosulphate solution; W = Weight in g of sample

d) Refractive Index (As per AOAC method 921.08, 2005 using Abbe refractometer)[27]: 2-3 drops of oil was placed on lower surface of prism. The temperature of refractometer was adjusted to 40 ± 0.1°C. The prisms was closed and tightened firmly with the screw-head and allowed to stand for 2 min and the refractive index was determined.

Results and Discussion

The results obtained for the various studies undertaken as described above are given below & discussed in details.

1) Effect of UV-C Radiation on Aflatoxin content and Fungal count in contaminated peanuts

The efficacy of UV-C irradiation for the elimination of aflatoxins and fungal count in peanuts was studied for different exposure time i.e. 2, 4, 6, 8, 10 and 12 h respectively for the specimen of peanuts placed at two different distances i.e. at 15 cm & at 30 cm [Fig I a & I b] from the source of UV-C light in two different experiments. The results of fungal count and aflatoxin content subjected to irradiation by UV-C light at 15 cm distance are presented in [Table I] and at 30 cm distance are presented in [Table II]. The decrease in the fungal count and aflatoxin content due to the effect of UV-C irradiation is also shown graphically in [Figure II and III] respectively. The results showed a gradual decrease in the fungal count and the aflatoxin content by increasing the time of irradiation. After irradiation for a period of 10 h, maximum reduction from 350 ppb for 0 h to 3 ppb for 10 h at a dose of 540 KJ/m^2 in aflatoxin content was observed. In the same period of irradiation, there was almost complete reduction (6 log) in fungal count for both the cases where specimens were kept at two different distances (15 cm and 30 cm) from the source of UV-C. The aflatoxin content was reduced to 3 ppb from 350 ppb with 99.1% reduction using UV-C dose of 540 KJ/m^{2}at a distance of 15 cm and to 9 ppb with 97.4 % reduction using UV-C dose of 360 KJ/m^{2}at a distance of 30 cm. Increasing the exposure time beyond 10 h however, did not indicate any further significant changes in either the aflatoxin content or the fungal count. Here, it may be noted that the reduced amount due to irradiation by UV-C upto 3 ppb is well within

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the MRL value prescribed as per the regulatory guidelines by USFDA and EU.[29, 30].

The results obtained are quite consistent with the results reported by several researchers earlier on the efficacy of UV-C irradiation in reduction of fungal count as well as mycotoxins. Murata et al.[31] had examined the effect of UV-C irradiation on mycotoxins such zearalenone (ZEN) and deoxynivanenol (DON) and established that UV radiation is effective in reducing mycotoxins both ZEN and DON in a time dependent manner both in solid form and in moist form. The use of UV and fluorescent light were investigated by Atalla et al.[32]for their ability to detoxify mycotoxins in wheat grains. The inoculated wheat grains were exposed to fluorescent light i.e. short and long wavelength of UV radiations and stored for three weeks under different conditions of relative humidity (50-80%) at ambient temperature and found complete elimination of only some mycotoxins such as aflatoxins and ochratoxins. Concentrations of mycotoxins were reduced to a varying extent depending on the relative humidity and period of illumination. Jubeen et al.[33] had investigated the effect of UV irradiation on aflatoxins in various types of nuts whereby the contaminated nuts were exposed to UV-C radiations of 265nm for 15, 30 and 45 min. The results reported from their study indicated that there was proportional decrease in aflatoxins level with increase in exposure time and though complete elimination of aflatoxin G_2 was achieved in all nut samples after an exposure of 15min, aflatoxin G1 showed 100% degradation only in almonds and pistachios and that too after longer period of exposure. Aflatoxin B1 showed maximum reduction of 96.5% in almond and pistachios after 45 min. Mazaheri[34] reported that UV radiations could decrease the concentration of aflatoxin B1 in pistachio. The results showed that the aflatoxin concentration decreased from 100 ppb to 78.189 ppb after 3 h and to 42.193 ppb after 13 h using UV radiation dose of 87.5μW/cm^2.

The reduction of fungal count with UV-C irradiation has been reported by several researchers [35, 36, 23]. Darvishi et al.[37]investigated the effect of UV-C radiation on Kurdistan' Strawberry where the strawberries were exposed to different doses (0.25 and 0.5 kJ/m^2) of UV-C (254 nm) radiation and reported a reduction of the yeast growth without affecting the sensory quality. Tandon et al.[38] investigated the effect of UV-C irradiation(14 mJ/cm^2 at wavelength 254 nm) on apple cider during storage and obtained an acceptable reduction in microbial loads. Escalona et al.[39] applied UV-C (0, 2.4, 7.2, 12 and 24 kJ/m^2) radiation to both sides of baby spinach leaves and found the reduction of microbial load during the storage of agriproduce on the shelf life. Similarly, the effect of UV-C radiation on microbial growth in vitro in *Monilinia fruticola* and in inoculated Yali pears (Pyrusbretschneideri Rehd.) were investigated by Zhang et al.[40]. They reported that spore germination of *M. fructicola* was significantly inhibited by each of the 3 doses (1, 5, and 10 kJ/ m²) in vitro and concluded that UV-C treatment could reduce postharvest disease by the germicidal and induced effects and maintain the quality by enhancing the antioxidant enzyme activities.

Although the exact mechanism of reduction or detoxification of aflatoxins by UV radiation remains unclear, it might be due to the disintegration of aflatoxin structure into less toxic or non-toxic fragments.

2) Effect of UV Radiation on Nutritional Parameters of Peanuts

Peanuts are characterized by their high oil and protein content as well as low carbohydrate content[41]. Peanut seeds are reported to contain 44 – 56 % fat, 22 – 30 % protein and 9 – 19.0% total carbohydrates[42, 43]. The values for the various nutritional parameters i.e. fat, protein and carbohydrate content of the unirradiated (control) and irradiated peanuts at both the distances i.e. 15 cm and 30 cm from the UV-C source are tabulated in [Table III] and are represented graphically in [Figure IV] and [Figure V] respectively. It was found that the irradiation did not have significant effect on the different nutritional parameters. The value for the protein content which was originally found to be 26.81 % remained almost the same after irradiation of the sample at both the distances. The carbohydrate content which was originally found to be 15.92% also remained the same after irradiation. However, the values for the fat content showed a slight decrease on irradiation. The values reduced from 48.32% to 44.50 % in the sample exposed at a distance of 15 cm from the UV-C source and reduced from 48.32% to 44.76% in the sample exposed at a distance of 30 cm from the UV-C source. This decrease could be due to the oxidative degradation of fat molecule on radiation exposure. Although the fat content shows a slight decrease but the values are well within the acceptable limits.

3) Effect of UV-C Irradiation on Physicochemical Properties of Peanut Oil

The effects of UV-C irradiation on physico-chemical parameters such as acid value, peroxide value, saponification value and refractive index of peanut oil extracted from peanut spread at a distance of 15 cm and 30 cm in individual experiments are summarized in [Table IV]. The values which were found to be 1.39, 9.19, 181.68 and 1.462 for acid value, peroxide value, saponification value and refractive index respectively in the oil extracted from irradiated peanuts did not showed much variation from the original values obtained from the oil extracted from un-irradiated peanuts. Our results are in agreement with the results of Amaral[44] that did not find significant alterations in quality parameters of fresh-cut melon irradiated with UV-C. Though there was a slight increase in the acid value was observed. The results are in agreement of Bachelliet al.[45], who reported that UV-C treatment on papaya juice increases the concentration of tritatable acidity. In contrast Perkins-Veazie et al.[46] on the blueberry showed that the titrable acidity was not affected by UV-C treatment.

Conclusion:

UV-C radiations of the wavelength varying from 254 – 265 nm is effective in reduction of bacterial load, yeast and mould count, fungal count and different types of mycotoxins on various types of food products. The efficacy of UV-C radiations depends upon the type of food product as well as the type of mycotoxins. Certain mycotoxins like

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aflatoxin G1 is found to be easier to decontaminate than the others. Likewise, it is difficult to decontaminate products like peanuts than the vegetables etc.

Even though the efficacy has already been established, the exact mechanism of the degradation has not been assigned as yet. Further, the studies on peanuts in India have been scanty and hence, the present study would be of use to the stakeholders of peanuts specially the regulatory agencies.

From the results obtained here in this study, it is evident that the two parameters are important to achieve the desired results from irradiation by UV-C; a) time of exposure or the dose of irradiation and b) distance from the source of UV-C (which again is an indicative of the irradiation dose). It has been found that for desired levels of reduction of aflatoxins, the source of radiation must be as close to the stock as possible. Further, in order to have a uniform effect, the peanuts must be spread evenly ensuring that the maximum surface of the nuts is exposed and there is no hindrance to the exposure by irradiation due to the layers of nuts.

It may also be noted here that for the lots of peanuts which are not yet contaminated with the aflatoxins but having significant level of yeast and mould count and likely to get contaminated on storage, irradiation by UV-C can be an effective technology for preventing the peanuts from getting contaminated with aflatoxins in future. This means that the UV-C irradiation cannot only be used for decontaminating the aflatoxin contaminated peanuts but its use is also a preventive measure to ensure that the peanuts do not get contaminated with aflatoxin or any other pathogenic microorganisms on storage.

Table 1: Results for effect of exposure time at 15 cm distance on aflatoxin content and fungal count in irradiated peanuts using UV-C light

Exposure time (h)	UV Dose (KJ/m^2)	Aflatoxin Content (ppb)	Fungal Count ($cfug^{-1}$)
Control	0	350 ± 2.8	$3.6 \times 10^6 \pm 0.55$
2	108	141 ; 138 ; 144 ; 142 ; 139 Mean : 141 ± 2.4	7.1×10^4 ; 7.9×10^4 ; 7.4×10^4; 7.8×10^4; 7.3×10^4 Mean : $7.5 \times 10^4 \pm 0.34$
4	216	24 ; 26 ; 25 ; 24 ; 23 Mean: 24 ±1.14	3.9 $\times 10^2$; 3.7×10^2 ; 4.2×10^2; 3.6×10^2 4.4×10^2 Mean : $3.9 \times 10^2 \pm 0.33$
6	324	17 ; 18 ; 18 ; 19 ; 17 Mean : 18 ± 0.84	2.2×10^2 ; 2.6×10^2; 1.9×10^2; 2.4×10^2 ; 1.5×10^2 Mean : $2.1 \times 10^2 \pm 0.43$
8	432	10 ; 8 ; 11 ; 9 ; 10 Mean : 10 ± 1.14	20 ; 22 ; 19 ; 18 ; 21 Mean :20 ± 1.58
10	540	3 ; 4 ; 3 ; 2 ; 3 Mean : 3 ± 0.71	< 10 ; < 10 ; < 10 ; < 10 ;< 10 Mean :< 10
12	648	2 ; 3 ; 3 ; 2 ; 3 Mean : 3 ± 0.55	N.D; N.D.; N.D.; N.D.; N.D. Mean : N.D

Table II: Results for effect of exposure time at 30 cm distance on aflatoxin content and fungal count in irradiated peanuts using UV light

Exposure time (h)	UV Dose (KJ/m^2)	Aflatoxin Content (ppb)	Fungal Count ($cfug^{-1}$)
Control	0	350 ± 2.8	$3.6 \times 10^6 \pm 0.55$
2	72	188 ; 194 ; 189 ; 191 ; 190 Mean : 190 ± 2.30	$2.5X\ 10^5$; 2.3×10^5; 2.0×10^5; 1.8×10^5; 1.9×10^5 Mean : $2.1 \times 10^5 \pm 0.29$
4	144	72 ; 74 ; 71 ; 67 ; 70 Mean : 71 ± 2.59	1.2×10^3 ; 1.4×10^3 ; 1.8×10^3; 1.7×10^3; 1.5×10^3 Mean : $1.5 \times 10^3 \pm 0.24$
6	216	33 ; 31 ; 36 ; 32 ; 35 Mean : 33 ± 2.07	3.9×10^3 ; 3.5×10^3 ; 3.2×10^3; 3.3×10^3; 3.6×10^3 Mean : $3.5 \times 10^3 \pm 0.27$
8	288	28 ; 25 ; 23 ; 22 ; 26 Mean : 25 ± 2.38	73 ; 77 ; 74 ; 71 ; 75 Mean : 74 ± 2.24

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10	360	9 ; 10 ; 8 ; 11 ; 9 Mean : 9 ± 1.14	< 10 ; < 10 ; < 10 ; < 10 ; < 10 Mean : < 10
12	432	8 ; 7 ; 9 ; 8 ; 9 Mean : 8 ± 0.83	N.D.; N.D.; N.D.; N.D.; N.D. Mean : N.D

1. Five different experiments were conducted; each at two different distances (15 cm & 30 cm) with different exposure time
2. Three replicate analysis were carried out for determination of aflatoxin content and fungal count for each of the fifteen experiments (3 x 5 = 15)
3. 5 replicate analysis were carried out for the control (un-irradiated) samples

Table III: Effect of different doses of UV-C irradiation on nutritional parameters of peanuts

S. No.	Exposure Distance (cm)	15 cm							30 cm						
	Exposure Time (h)	0	2	4	6	8	10	12	0	2	4	6	8	10	12
	UV Dose (KJ/m²)	0	108	216	324	432	540	648	0	72	144	216	288	360	432
	Parameters Studied														
1	Fat Content (%)	48.32 ± 2.51	47.63 ± 2.14	46.48 ± 1.98	45.46 ± 2.07	45.20 ± 2.67	44.91 ± 2.35	44.50 ± 2.22	48.32 ± 2.51	48.16± 2.19	47.95 ± 2.32	46. 65± 2.04	45.56 ± 1.65	44.31 ± 1.47	44.76 ± 2.54
2	Protein Content (%)	26.81± 0.63	26.56± 0.57	26.70 ± 0.71	26.35 ± 0.38	26.92 ± 0.49	26.45 ± 0.65	25.25 ± 0.54	26.81± 0.63	26.65 ± 0.32	26.74 ± 0.29	26.96 ± 0.43	26.21 ± 0.58	26.48 ± 0.21	26.12 ± 0.25
3	Carbohydrate Content (%)	15.92 ± 0.65	15.47 ± 0.71	15.11 ± 0.52	15.34 ± 0.31	15.26 ± 0.45	15.28 ± 0.29	14.98 ± 0.37	15.92 ± 0.65	16.25 ± 0.31	15.62 ± 0.51	15.51 ± 0.27	15.42 ± 0.19	15.38 ± 0.16	15.08 ± 0.25

1. Zero hour sample is the control sample i.e. without irradiation
2. All results mentioned are mean of 5 replicate analysis

Table IV: Effect of different doses of UV-C irradiation on physicochemical parameters of peanut oil

S. No.	Exposure Distance (cm)	15 cm							30 cm						
	Exposure Time (h)	0	2	4	6	8	10	12	0	2	4	6	8	10	12
	UV Dose (KJ/m2)	0	108	216	324	432	540	648	0	72	144	216	288	360	432
	Parameters Studied														
1	Acid Value (mg KOH/g of oil)	1.39 ± 0.04	1.45 ± 0.06	1.54 ± 0.05	1.87 ± 0.10	2.10 ± 0.08	2.32± 0.07	2.46 ± 0.05	1.39 ± 0.04	1.35 ± 0.05	1.40 ± 0.09	1.62 ± 0.07	1.65 ± 0.08	1.70 ± 0.05	1.73 ± 0.02
2	Peroxide Value (mEq/kg)	9.19± 0.83	8.93 ± 0.91	8.47 ± 0.75	8.59 ± 0.52	8.92 ± 0.58	9.08 ± 0.69	9.24 ± 0.56	9.19± 0.83	9.01 ± 0.78	9.34 ± 0.61	9.89 ± 0.55	9.05 ± 0.42	9.22 ± 0.25	9.17 ± 0.31
3	Refractive Index, 40°C	1.462 ± 0.001	1.462 ± 0.001	1.463 ± 0.001	1.462± 0.001	1.463 ± 0.001	1.462 ± 0.001	1.463 ± 0.001	1.462 ± 0.001	1.462 ± 0.001	1.462 ± 0.001	1.463 ± 0.001	1.462 ± 0.001	1.463 ± 0.001	1.463 ± 0.001
4	Saponification Value (mg KOH/g of oil)	181.68 ± 0.32	181.73 ± 1.10	182.45 ± 0.91	182.13 ± 0.12	182.72 ± 0.52	182.43 ± 0.45	182.56 ± 0.65	181.68 ± 0.32	180.29 ± 0.73	181.51 ± 0.34	181.92 ± 0.57	182.10 ± 0.86	182.31 ± 0.36	182.27 ± 0.66

1) Zero hour sample is the control sample i.e. without irradiation
2) All results mentioned are mean of 5 replicate analysis

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Figure I a and I b: Exposure of contaminated peanuts to UV-C irradiation: Sample tray placed at a distance of 15 cm from UV-C tube; Sample tray placed at a distance of 30 cm from UV-C tube

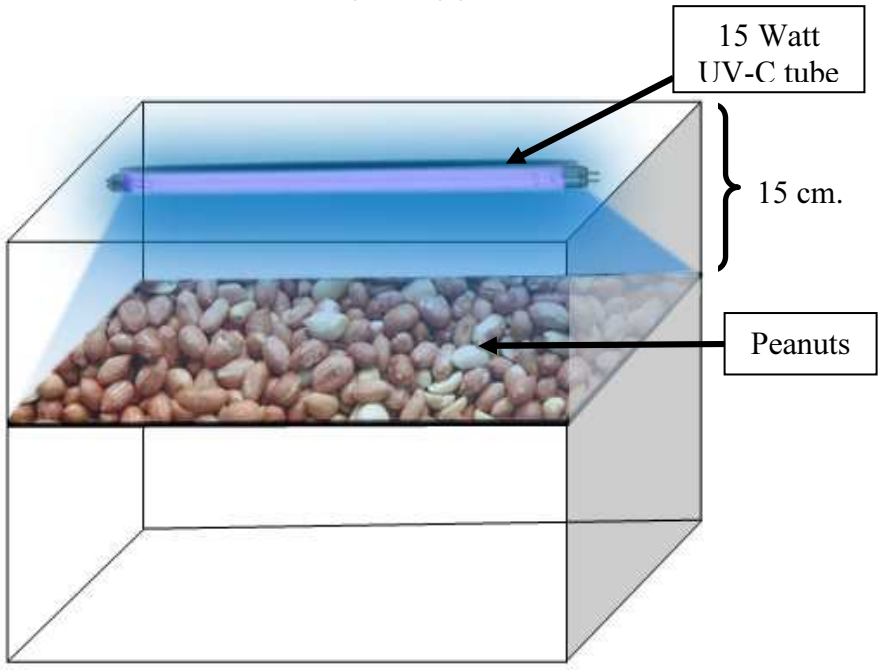

Figure 1 a

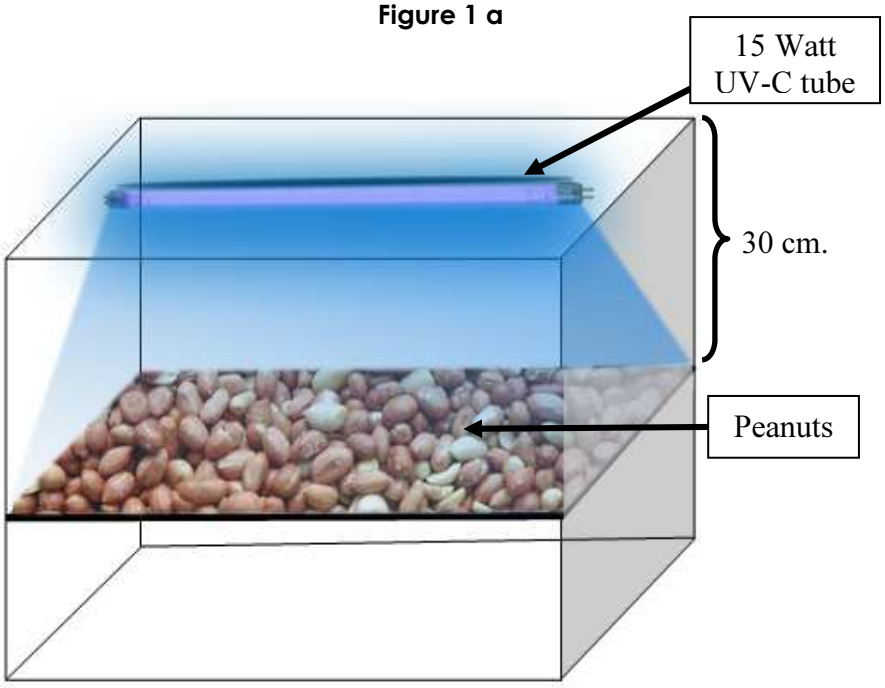

Figure 1 b

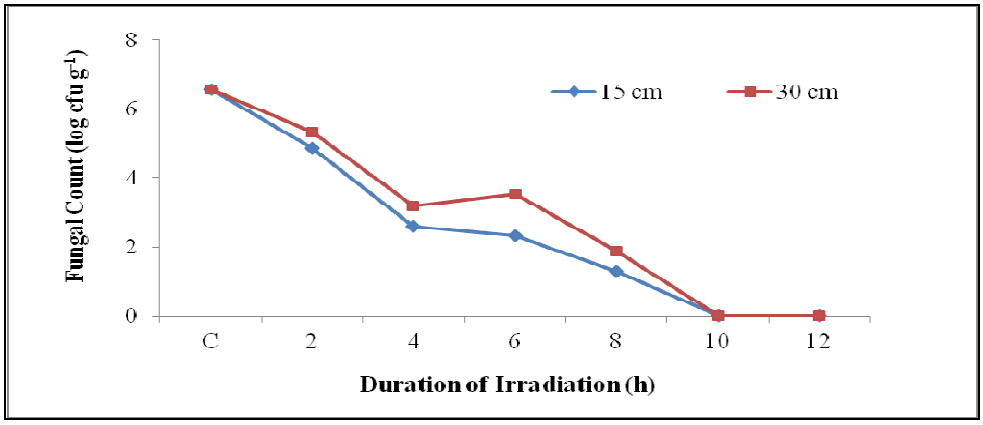

Figure II Effect of UV-C irradiation on fungal count of peanuts irradiated at a distance of 15 cm and 30 cm from the UV-C source

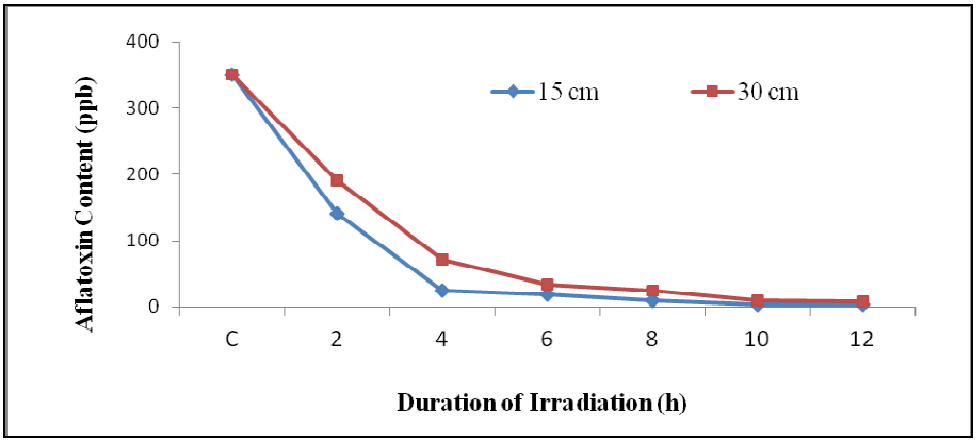

Figure III Effect of UV-C irradiation on aflatoxin content of peanuts irradiated at a distance of 15 cm and 30 cm from the UV-C source

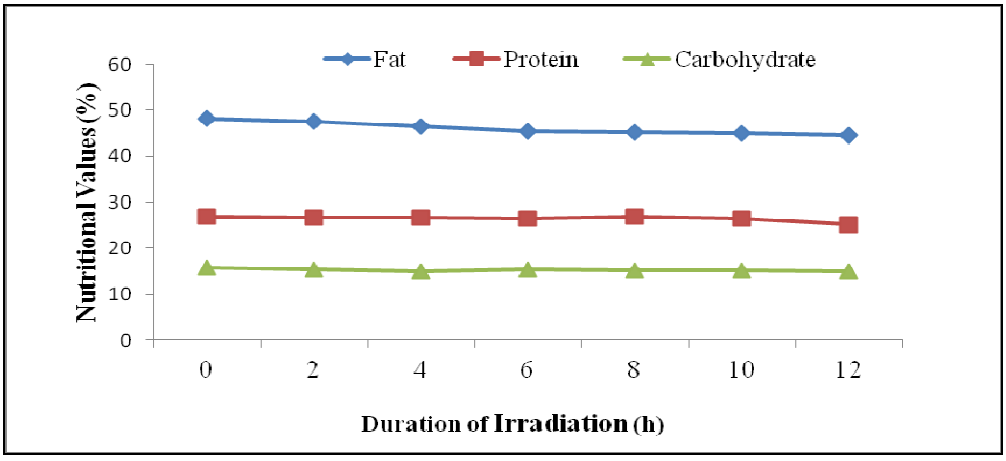

Figure IV Effect of UV-C irradiation on Nutritive values of Peanuts at a distance of 15 cm from the UV-C source

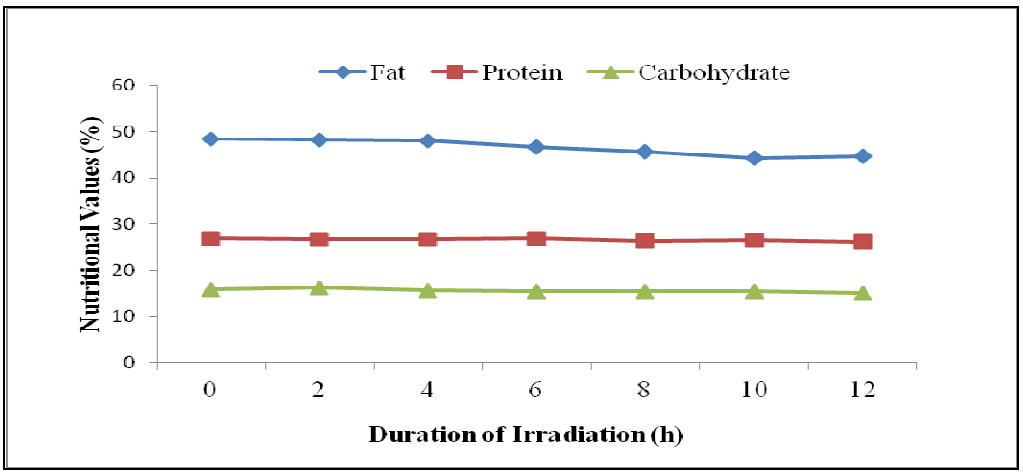

Figure V Effect of UV-C irradiation on Nutritive values of Peanuts at a distance of 30 cm from the UV-C source

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